The Breast 19 (2010) 489–492

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

Evaluation of HER2/neu oncoprotein in serum and tissue samples of women with breast cancer: Correlation with clinicopathological parameters

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ARTICLE INFO

Article history:
Received 6 August 2009
Received in revised form
3 May 2010
Accepted 24 May 2010
Available online 2 August 2010

Keywords:
Serum HER2/neu
Breast carcinoma
Immunohistochemistry
ELISA

ABSTRACT

Background: HER2/neu (HER2) is a proto-oncogen of the EGF Receptor family. The assessment of serum HER2 level is useful for predicting the patients' response to chemotherapy or hormonal therapy and selection of proper patients for treatment with Herceptin.
We aimed to compare serum HER2 levels with immunohistochemistry in tumoral tissues and investigate correlation between these levels and various prognostic factors.
Materials and methods: This cross-sectional study was conducted on 75 patients with breast carcinoma referred to surgical ward of Mashhad Imam Reza's hospital from November 2008 to February 2009. Pre-operative serum samples were collected and stored in −20 °C.
Surgical samples were investigated for the type of carcinoma, tumor size, lymph node metastasis, stage as well as grade of the tumor. Tissue HER2 over-expression was evaluated by immunohistochemistry (IHC) staining and HER2 levels were studied by ELISA method. Statistical analysis was performed by SPSS software.
Results: Serum HER2 cut-off level was 18.4 ng/ml; 46.7% of patients were serum HER2-positive and 43% were IHC positive. There was a high statistical correlation between these two parameters ($P = 0.018$). Statistically, there was no significant correlation between serum HER2 and age, tumor size, stage, grade and metastatic lymph nodes ($P > 0.05$).
Conclusion: Serum HER2 level assay can be considered as a complementary method besides tissue methods.

Introduction

The human epidermal growth factor receptor-2 proto-oncogene, also known as HER2 and c-erbB-2, encodes a growth factor receptor which has been found to play an important role in breast cancer. In up to 30% of breast cancer patients, the HER2 gene is amplified and its associated receptor protein is over expressed on the tumor cell surface, thus playing an important role in the malignant transformation and clinical aggressiveness of breast cancer.[1]

HER2 expression can be effective in the evaluation of prognosis[2] and novel treatment methods include gene therapy, immunotherapy, immunotoxins and tyrosine kinase inhibitors.[3–6]

Immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) are reliable ways to identify over-expression or amplification of the HER gene, but each technique requires a high-quality tissue sample, which may not be available.

In recent reviews, Carney et al. found the prevalence of elevated HER2 levels to be between 0% and 38% (mean, 18.5%) in women with primary breast cancer.[7]

In this study we evaluated the concordance of serum HER2 levels with immunohistochemistry in tumor tissue, and examined the relationship between serum HER2 level with several clinicopathological features of patient samples including grade and stage of tumor, tumor size, involvement of axillary lymph nodes and patient age.

Materials and methods

Patients and samples

In this study, primary statistical community included patients admitted to the surgery ward of Imam Reza hospital from November 22, 2008 to February 18, 2009. All patients who were

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doi:10.1016/j.breast.2010.05.012

considered for surgery of breast mass were evaluated using medical records, and cases with the history of malignancies other than breast carcinoma were excluded from the study. A serum sample was drawn from patients before surgery and was kept frozen in microtubes. After surgery and evaluation of sample pathology, benign cases were excluded. Altogether 75 patients were included into the study. First, tissue samples were stained by Haematoxylin and Eosin (H&E) method, then carcinoma type was determined and graded using Bloom–Richardson staging system. Lymph nodes were also evaluated for metastasis.

Immunohistochemistry

Paraffin-embedded tissue was cut into 4-μm sections and were deparaffinized in xylene and rehydrated through alcohols to distilled water range. Then polyclonal Rabbit Anti human c-erb-2 oncoprotein (code No.A 0485 Dakocytomation Denmark) at dilution of 1:250–1:350 was used to estimate over-expression of HER2. According to the Hercep Test TM criteria, the samples were divided into four groups of 0, +1, +2 and +3 according to staining extent. Among the above groups, 0 and +1 were regarded as negative and +2 and +3 as positive immunohistochemically.

Serum HER2 extracellular domain (ECD) assay

Five milliliters of peripheral blood were collected in a sterile test tube and after 30 min centrifuged at 3000*g* for 10 min at room temperature. Serum samples were stored at −20 °C till the time of the assay. After collection of all samples, to measure the serum HER2/ECD concentration, we used enzyme-linked immunosorbent assay method (ELIZA Kit, Bender Medsystem kit sp HER2 BMS207). The limit of detection was 0.06 ng/ml and intra- and inter-assay coefficients of variation were 1.9% and 5.8%, respectively.

Statistical analysis

Immunohistochemical staining and serum HER2 results together with clinicopathological information were analyzed using SPSS software. The χ^2 test was used to assess the association between clinical and pathological features and the level of expression of HER2/ECD. We used the Kendall's test to compare the serum HER2 concentration with tissue HER2 status.

Results

The age range of the patients was 26–72 years with an average of 46. Based on an initial study performed on 20 normal samples and comparison of the results with information from ELISA kit, association between serum level cut-off was 18.4 ng/ml, with the following results (Table 1).

Thirty-five samples were positive for HER2 in serum and 40 samples were negative (53.3%). The serum values ranged between 7.2 and 96 ng/ml with an average of 24.9 ng/ml.

In immunohistochemistry staining for HER2, 42 cases (57%) had negative staining and 32 cases (43%) were positive. It should be noted that one case was omitted from the study due to technical issues. There was a significant between positive and negative results of immunohistochemistry and HER2 serum level ($P = 0.008$) using Kendall's test. (Table 2, Fig. 1).

The diameter of the tumor was between 1 and 7 cm. The results of *t*-test showed no correlation between tumor size with presence or absence of HER2 in serum ($P = 0.862$).

Histological samples of patients were graded based on Bloom–Richardson staging system to 1, 2 and 3 grades. Using Kendall's test, tumor stage has no correlation with presence or absence of HER2 in 95% confidence level ($P = 0.076$), but it has significant correlation in 90% confidence level. There was no correlation between pathologic stage of tumor and the presence or absence of HER2 in serum (Kendall's test, $P = 0.865$).

There was no correlation between the number of axillary metastatic lymph nodes (49.3% of cases) and the presence or absence of serum HER2 ($P = 0.297$).

According to carcinoma histology, 68 cases had invasive ductal type, two cases comedocarcinoma, one case tubular, one case metaplastic, two cases lobular and one case had medullary carcinoma. In addition to ductal carcinoma, comedocarcinoma and tubular carcinoma had high HER2 levels while others were negative.

Table 1
Clinicopathological features of 75 patients with breast cancer.

Serum HER2-negative cases	Serum HER2-positive cases	Number = 75	Criteria	
36(54%)	32(46%)	68	Invasive ductal carcinoma	Tumor histology type
–	1	1	Invasive tubular carcinoma	
–	2	2	Comedocarcinoma	
2	–	2	Invasive lobular carcinoma	
1	–	1	Medullary carcinoma	
1	–	1	Metaplastic carcinoma	
53.3%	46.7%	24.9 (7.2–96)	HER2 (ng/ml)	Mean serum level
50%	50%	8 (11.4%)	Grade 1	Tumor grade[a]
59%	41%	46 (65.7%)	Grade 2	
25%	75%	16 (22.8%)	Grade 3	
–	100%	2 (2.6%)	Stage 0	Stage
42%	58%	12 (16%)	Stage 1	
66%	34%	41 (54.6%)	Stage 2	
40%	60%	20 (26.6%)	Stage 3	
46%	54%	37 (49.3%)	Positive	Axillary lymph nodes metastasis
58%	42%	38 (50.6%)	Negative	

[a] Five cases had non-invasive ductal carcinoma and are not included.

Discussion

HER2 is a 185 kD integral membrane protein. Extracellular domain of HER2 is broken by metalloproteases and detached from other parts of HER2 and enters the blood circulation.[8]

Real-time PCR and ELISA are true quantitative methods, whereas IHC and FISH are morphologic in situ and semi-quantitative tests. Moreover, the ELISA for serum HER2 is a dynamic test that can be performed at any time and can be used when primary tumor samples are unavailable, and eliminates the need for biopsy. ELISA could also be helpful when tissue results are discordant.[9]

Table 2
Frequency distribution of positive and negative immunohistochemistry and serum HER2 levels.

			IHC		Total
			Negative	Positive	
Serum HER2	Negative	Count	27	12	39
		% Within IHC	64.3	37.5	52.7
	Positive	Count	15	20	35
		% Within IHC	35.7	62.5	47.3
Total			42	32	74
			100%	100%	100%

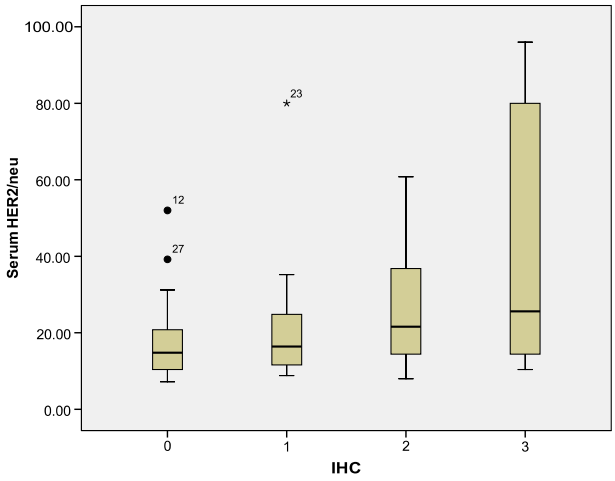

Fig. 1. Box plot diagram of IHC staining intensity and HER2 serum level.

In this study after determining HER2 serum levels in 20 serum samples of normal individuals and normal distribution of resulting data, serum HER2 cut-off was found to be 18.4 ng/ml, approximately similar to the kit cut-off value. This value has been found to be between 8.83 and 15 ng/ml in other studies[8,10,11] due to the use of different kits in the other studies and variation of sensitivity and specificity of the kits.

An immunoassay for serum HER2 that was recently cleared by the US Food and Drug Administration has a cut-off of 15 ng/ml which is very close to the optimal value determined in our study (18.4 ng/ml).

A previous report also showed mean serum HER2 of 22.2 ng/ml in the tissue HER2-negative group, which was significantly lower than the concentration of 363 ng/ml in the tissue HER2-positive group. They determined that 37 ng/ml serum HER2 showed 95% specificity and 62% sensitivity in predicting tissue HER2, and they suggested that this value can be used as the cut-off because they focused on a cut-off concentration at which the false positive rate for tissue positivity was low.[12] It is noteworthy that in majority of the studies, the cut-off determined by kit producer was used, and it was established by Kushlinskii through studying healthy individuals.[11]

In our study, there was no correlation between age and serum level of HER2. In the former studies by Slamon (1978),[13] Dati (1991),[14] Ali (1988),[15] Guerin (1988)[16] and Clark (1991)[17] there was no significant correlation between age and serum HER2 level either. The only point in our study was presence of 10 patients (13.3% of patients) with less than 35 years of age which may indicate screening on lower age groups.

As expected, the majority of carcinomas were of ductal type. Among other types, two cases of comedocarcinoma had serum HER2 levels of 80 and 37.6 ng/ml. There was also one case of tubular carcinoma with serum level of 60.8 ng/ml which was regarded as positive, but other carcinomas had serum levels lower than 18.4 ng/ml and were considered negative. The above findings are in accordance with the results of textbooks indicating high HER2 expression in nearly all cases of comedocarcinoma (20–30% of invasive ductal carcinoma and a lower percentage of invasive lobular carcinoma).

As expected, in most cases the tumor was in external upper quadrant of breast, mainly on the left side, but there was no correlation between tumor location and serum HER2 level ($P = 0.206$). This issue has not been evaluated before in the literature.

In this study, performed on patients with primary breast cancer in stages I–III, 46.7% of patients had serum levels higher than 18.4 ng/ml and were considered positive. Serum level range of HER2 was between 7.2 and 96 with an average of 24.9 ng/ml. Previous studies have reported a range of values for serum HER2 level, from 18.9%[18] to 79%.[8] Also, the range of serum HER2 level varies from 2.3 to 23.3 (mean of 4.8 ng/ml) in Imoto's study[19] to Hudelist's study with 5.2–6072 range and mean of 53.7 ng/ml.[10] This wide range of results could be due to the following reasons.

- Patient selection: in some studies, stages I–III and in other patients with distant metastases have been selected. On the other hand, some other malignancies such as prostate, pancreas, bladder and lung cancer can cause elevated serum HER2 levels which can be falsely ascribed to breast carcinoma if not considered enough.
- Sample number: the results will be more valid with larger sample size.
- Experiment kit: difference in kits and their specificity and sensitivity is a main reason for variation in research results. If a kit has high sensitivity and low specificity, its results will have a lot of false positive results and vice versa.

In our study, 43% of patients (32 cases) had positive immunohistochemistry (+2 or +3 staining intensity). In previous studies, 20–30% of patients had positive IHC.[2] Results resembling ours have been reported by Yang (1997),[20] Abadjian (1996)[21] and Kushlinskii (2007)[11] citing IHC positive cases as 50%. Statistical analysis of our results showed strong correlation of serum HER2 level with positive and negative immunohistochemistry cases and staining intensity ($P = 0.018$). A study on patients with metastatic breast cancer showed that 64% of cases had HER2 in serum and 50% were IHC positive. There was also a significant statistical correlation between these cases.[11]

As shown in diagram and table of comparison of positive and negative immunohistochemistry and serum HER2 level, there are cases of disparity of positive and negative cases in both groups. There are several possible explanations for such discrepancies between the HER2 status of primary breast tumors and serum HER2 concentrations. This can be due to technical problems of each of these methods, need for personal interpretation in IHC method, the effect of dilution in ELISA results and expected differences due to change in specificity and sensitivity of these methods. Clonal changes between the primary breast tumor and distant metastases may also affect HER2 status. Patients with negative tumor HER2 status at the time of biopsy may become HER2-positive as they progress to metastatic disease. This could explain discrepancies between negative tissue testing and increased serum HER2 concentrations in metastatic breast cancer. Moreover, reduced metalloprotease activity could lead to decreased serum HER2 concentrations.[9]

In our study, no correlation was found between tumor size and serum HER2 levels. This lack of correlation was also present in Molina (1992)[22] and Slamon's studies[13] but Rilke (1991)[23] and Van de vijver(1988)[24] showed that serum HER2 level increased with increase in tumor size. Variation in tumor necrosis or concomitant presence of lymphatic or vascular invasion could be reasons for such differences. In our study, the relationship between tumor grade and serum HER2 level has been consistent in 90% confidence level, but not in 95% ($P = 0.076$). While Molina, Rilke and Gullick have reported no link between tumor grade and serum HER2 level,[22,23,25] Choi (2003) and Imoto (2007) reported 95% correlation.[26,19]

In this study, there was no correlation between pathological tumor stage and serum HER2 level ($P = 0.865$). In two former studies by Yuan and Imoto correlation between these two parameters has been reported.[18,19] In Kushlinskii's study lack of correlation has been emphasized.[11]

In this study, 49.3% of patients (37 cases) had metastatic involvement of axillary lymph nodes, but there was no significant

relationship between the number of involved lymph nodes and serum HER2 level ($P = 0.297$). Other studies have also reported contradicting results: Yuan's study reported lack of relationship while Imoto showed significant correlation indicating increased serum HER2 level by increase in the number of metastatic lymph nodes.[18,19]

Because of higher specificity and sensitivity of ELISA test relative to immunohistochemistry,[27] it is recommended to use serum HER2 level measurement as complementary of histological methods. According to this study, we suggest to evaluate serum HER2 level first and if it was negative then proceed to HER2 expression evaluation in tissue sample immunohistochemically.

Conflict of interest

The authors have no conflict of interest.

Acknowledgements

The authors are grateful to Mr. Parviz Maroozi for his assistance in statistical analysis.

The results described in this paper was part of a pathology student thesis proposal and was supplied financially by a research grant (No. 84337) from the vice chancellor for research of Mashhad University of Medical Sciences, Mashhad, Iran.

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